REMARKS

Applicants would like to thank Examiner Kim for the phone conferences held on March 8 and 13, 2006. During the phone conference March 13, 2006, Examiner Kim stated that she will most likely withdraw the rejection under 35 U.S. C. §112, first paragraph (new matter) if we submitted our arguments in writing because she found support for the amendment. Examiner Kim also stated that she will consider our written arguments for vacating the finality of the Office Action.

Premature Finality of the Office Action

Applicants respectfully submit that the finality of the Office Action is premature. The Office Action introduced new grounds of rejections under 35 U.S.C. §§ 102(b) and 103(a) that were not necessitated by Applicants' amendment. In the Response of November 8, 2005, claim claim 106 replaced canceled claim 71. The difference between claims 106 and canceled claim 71 is the insertion of "wherein pretreatment comprises applying means for enhancing penetration and/or barrier disruption of the skin" in line 3, of claim 106. Claim 106 does not recite new limitations necessitating the new grounds of rejections. Without acquiescing to the propriety of the new grounds of rejections, Applicants respectfully point out these rejections should have been introduced in the First Office Action over claim 71 and its dependent claims, if the Patent Office considered them to be proper rejections.

Since the Patent Office introduced these new grounds of rejections under 35 U.S.C. §§ 102(b) and 103(a) in a Second Office Action, the Second Office Action should have been a Non-Final Office Action because they are new grounds of rejections not necessitated by Applicants' Amendments. Thus, Applicants respectfully request withdrawal of the finality of the Office Action.

Status of the Claims

Claims 106-159, and 161 are currently pending in the present application. Claims 1-105 and 160 have been canceled without prejudice or disclaimer of the subject matter claimed

therein. Claims 106-110, 142, and 161 have been amended.

Amendments to the Claims

Claims 106-110, 142, and 161 have been amended. These amendments do not introduce prohibited new matter.

Claims 106-110 have been amended for consistency of claim language. Support for the amendments to these claims can be found in claim 106.

Claim 142 has been amended to supply separate specific embodiments of the claimed invention. Representative support for the amendment to claim 142 can be found in canceled claim 160, on page 31, lines 8-15, and in Example 12 (pages 60-62).

Claim 161 has been amended to be consistent with the amendment to claim 142.

Rejections of the Claims Under 35 U.S.C. § 112, First Paragraph

A. Claims 142-161 are rejected under 35 U.S.C. § 112, first paragraph, because they contain subject matter which was not described in the specification (New Matter rejection).

The Office Action alleges that the specification does not provide written description for the phrases "wherein said area of the skin comprises a draining lymph node field" and "to the same draining lymph node field" recited in claim 142. Applicants respectfully point out that representative support for these phrases can be found on page 8, lines 3-5, page 10, lines 12 and 13, and page 31, lines 8-15 of the specification. Accordingly, the recitation of these phrases does not constitute new matter. During the phone conference of March 13, 2006, Examiner agreed that there is support for these phrases.

However, in the interest of compact prosecution, Applicants have amended claim 142. Claims 143-159 and 161 are dependent upon claim 142. Thus, the rejection is not applicable to claims 142-161.

B. Claims 142-161 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement.

The Office Action alleges that the specification does not enable a method of inducing an

immune response comprising applying a formulation to more than one application site and to a site overlying more than one draining lymph node and cites the factors of *In re Wands* in support of its position.

Claims 142 to 161 are directed to a method for inducing an antigen-specific immune response in a subject comprising delivering parenterally a first formulation containing an antigen to a subject; pretreating an area of the skin of a subject; and applying a second formulation comprising an adjuvant to the area of the skin.

The specification describes in detail multiple applications of formulations comprising antigen and/or adjuvant to an area of skin (pages 8, lines 6-19; page 31, lines 12-15; and Examples 12, 18, and 20). Specifically, the specification discloses parenteral delivery of a formulation followed by transcutaneous immunization to boost or prime an immune response induced by parenteral delivery (page 8, lines 11-15 and page 31, lines 12-15). As an example, Example 12 teaches intramuscular injection of DT to the hind thigh of mice followed by transcutaneous immunization of DT toxoid and CT, as the adjuvant, to the back of mice. Table 12 summarizes the results of Example 12 and shows that transcutaneous immunization is useful in boosting or priming an immune response induced by other routes of delivery.

Moreover, Frech *et al.* confirm that an adjuvant, such as LT, administered as an immunostimulant patch on the skin subsequent to an influenza vaccination, improved influenza immune responses in the elderly (see attached, Frech *et al.* Vaccine 23 (2005) 946-950). Frech *et al.* report that elderly adults who received intramuscular injection of an influenza vaccine containing HA followed by an LT patch placed 5 cm distal to the vaccine injection site showed enhanced immune response as compared to those elderly who only received vaccine injection alone (see Frech *et al.*: abstract, page 947, left column (last paragraph), Table 2). The report of Frech *et al.* supports the claimed invention of parenteral delivery of an antigen followed by application of a formulation comprising at least one adjuvant to the skin to enhance the immune response induced by the antigen.

Accordingly, given the teachings and data disclosed in the specification, the claimed invention is not unpredictable. A person of ordinary skill in the art would be able to make and

use the claimed invention without undue experimentation. The experimentation necessary to practice the claimed invention is only routine. Therefore, the specification provides sufficient guidance in the description of the invention and disclosed examples to enable a person of ordinary skill in the art to make and use the claimed method for inducing an immune response, in the absence of evidence to the contrary.

Applicants respectfully point out that the initial burden is on the Patent Office to provide a reasonable explanation as to why the scope of protection provided by the claims is not adequately enabled by the disclosure. *In re Wright*, 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). Moreover, the court in *In re Marzocchi* stated that it is incumbent upon the Patent Office to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up its assertions with acceptable evidence or reasoning which is inconsistent with the contested statement. *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971). The Office Action has not provided a reasonable explanation as to why the scope of the claims is not adequately enabled by the specification and has not provided evidence showing that the claims are not enabled by the specification.

Rejection of the Claims Under 35 U.S.C. § 102(b)

Claims 106, 107, 109, 110, 114-121, 124, 125, 127, 128, and 132-129 are rejected under 35 U.S.C. § 102(b) as being anticipated by WO 95/17211.

As discussed above, this is a new ground of rejection. Applicants' amendment to claim 106 in the previous response, submitted November 8, 2005, did not necessitate this new ground of rejection.

Claims 106 and 124 are directed to a method of inducing an antigen-specific immune response comprising pretreating an area of the skin of a subject and applying a formulation to the treated area of the skin of a subject. Claims 107, 109, 110, 114-121, 125, 127, 128, and 132-129 are dependent upon claims 106 and 124.

WO 95/17211 does not teach a method of inducing an antigen-specific immune response by applying a formulation to an area of the skin of a subject. The cited reference only teaches

applying a formulation to mucosal surfaces of an organism, for example, oral or intranasal delivery of formulations comprising antigen and adjuvant, as discussed on page 1 lines 29-31 of the cited reference. In contrast, the presently claimed method involves transcutaneous immunization which comprises delivery of antigens and adjuvants through the skin.

Delivery of antigens and adjuvants through the skin is different from delivery through mucosal surfaces because mucosal surfaces and the skin are structurally and functionally distinct tissues. The mucosa is a moist semi-permeable mucous membrane that lines hollow organs or body cavities, such as the nasal cavity, the digestive tract, and the oral cavity. The mucosa consists of epithelium and lamina propria. As an example, Wheater *et al.* show in Fig. 11.2 that the nasal mucosa consists of pseudostratified, columnar, ciliated epithelium, with numerous goblet cells supported by a vascular lamina propria containing serous and mucous glands. (see attached, Wheater *et al.*, Functional Histology, page 163, Churchill Livingstone (1979)). The major functions served by the mucosa are protection, absorption and secretion. In the case of the nasal mucosa, the mucus secreted by the goblet cells and mucous glands, traps air particulates, and the ciliated epithelium propels the mucus with the trapped particles towards the pharynx where it is swallowed and inactivated in the gastro-intestinal system. *Id.* In the case of the small intestine, the epithelial lining not only protects itself from noxious intestinal contents by the secretion of mucus, but also is involved in absorption of the products of digestion. *Id.* at 49.

On the other hand, the skin is the largest organ of the body and forms the continuous external surface of the body. The skin consists of an external surface called the epidermis comprising a keratinized squamous epithelium; the dermis comprising fibro-elastic connective tissues supporting the epithelium; and the hypodermis or subcutaneous layer, a layer of loose connective tissue containing variable amounts of adipose tissue, underlying the dermis. *Id.* at 116. One of the functions of the skin protection against UV light and chemical, thermal, and mechanical insults. Thus, structurally unlike the mucosa, the surface of the skin contains a keratinized epithelium, and functionally, unlike the mucosa, the skin has an impermeable surface to prevent dehydration and acts as a barrier to invasion by microorganisms. *Id.*

Moreover, the surface of the mucosa consists of simple epithelia which are almost always

found on absorptive and secretory surfaces. *Id.* at 50. The cells of the simple epithelia range in shape from extremely flattened to tall columnar depending on their function. Figures 4.1-4.3 show that the simple squamous epithelium is composed of flattened irregularly shaped cells and is involved in passive diffusion of gases in the lungs or fluids in the walls of the capillaries. *Id.* Figures 4.4-4.7 show the simple cuboidal epithelium lining the collecting ducts of the kidney, salivary glands and pancreas, and the simple columnar epithelium lining the small intestine and stomach. The simple epithelium provides little protection against mechanical abrasion. *Id.* On the other hand, the epithelium of the skin is a specialized form of stratified squamous keratinized epithelium. *Id.* at 54 and 116 (Figs. 4.14 and 8.1). This epithelial surface is adpated to withstand constant abrasion and dessication to which the body is exposed. *Id.* at 54. Therefore, the skin and the mucosa are structurally distinct tissues.

Further, the cited reference discloses the need for a non-toxic mucosal adjuvant for delivery of antigens to mucosal surfaces as discussed in detail on pages 3-5. The cited reference specifically states, "wild-type CT is extremely toxic as a mucosal adjuvant to humans, rendering the use of CT having any substantial residual toxicity as a mucosal adjuvant in humans entirely out of the question" (page 3, lines 6-9). In contrast, as discussed on page 2, lines 11-22 of the specification, CT placed on skin is non-toxic, while injection of CT into the skin results in swelling and redness, and large doses of LT placed on the skin of humans have been shown to induce a systemic immune response without local or systemic toxicity. Accordingly, a toxic mucosal adjuvant is non-toxic when applied to the skin and can be used in the presently claimed invention.

Also, the presently claimed method requires treating the skin to enhance penetration or barrier disruption either prior to or at the same time as applying the formulation. Applicants respectfully submit that the cited reference does not disclose treating the mucosal surface prior to or at the same time as delivery of antigen.

In summary, the cited reference does not disclose the limitations required in the claims. The cited reference neither teaches delivery of an antigen through the skin nor discloses treating the skin prior to or at the same time as application of the antigen. Also, the cited reference

requires the use of non-toxic mucosal adjuvants with the antigen for delivery to the mucosal surface. In contrast, a toxic adjuvant, such as CT, that cannot be used for mucosal delivery can be applied to the skin for transcutaneous immunization, the method of the present invention. Thus, the cited reference does not anticipate the claimed invention.

Rejection of the Claims Under 35 U.S.C. § 103

Claims 106-108, 113, 122-126, 131, 140 and 141 are rejected under 35 U.S.C. § 103 as being unpatentable over WO 95/17211, in view of U.S. Patent 4,810,499 ('499).

As discussed above, this is a new ground of rejection. Applicants' amendment to claim 106 in the previous response, submitted November 8, 2005, did not necessitate this new ground of rejection.

Claims 106 and 124 are directed to a method of inducing an antigen-specific immune response comprising treating an area of the skin of a subject and applying a formulation to the treated area of the skin of a subject. Claims 107, 108, 113, 122, 123, 125, 126, 131, 140, and 141 are dependent upon claims 106 and 124.

The deficiencies of WO 95/17211 are discussed immediately above under the previous rejection. Delivery of antigens through the mucosal surface does not render obvious the delivery of antigen through the skin of a subject because the mucosal surface is structurally and functionally distinct from the skin, as discussed in detail immediately above. Moreover, the delivery of antigens/adjuvants through the mucosal surface is different from delivery through the skin because the present inventors have unexpectedly found that toxic mucosal antigens/adjuvants are non-toxic when applied to the skin, as discussed above and on page 2, lines 11-22 of the specification.

Further, it has been reported that intranasal influenza vaccine containing LT approved for distribution and use in Switzerland has been shown to cause Bell's palsy and has been withdrawn from clinical use (see attached, R. Couch, 2004, N. Engl. J. Med., 350(9): 860; Mutsch *et al.*, 2004, N. Engl. J. Med. 350(9):896). These reports teach away from the claimed invention of using antigens/adjuvants, such as HA/LT (Example 18) and CT/Hib-PS (Example 19), for

transcutaneous immunization. Accordingly, the delivery of antigens/adjuvants to the mucosal surface, such as the nasal cavity, as disclosed by WO 95/17211, does not render the claimed invention obvious.

U.S. Patent '499 is relied upon for disclosing transdermal delivery. However, U.S. Patent '499 does not cure the deficiencies of WO 95/17211. U.S. Patent '499 does not teach transdermal delivery of formulations comprising antigen and adjuvant that induce an antigen-specific immune response. U.S. Patent '499 only discloses transdermal delivery of chemicals which are small molecules. Transdermal delivery of small molecules is well known and routinely practiced, but is not analogous to transdermal delivery of antigen and adjuvant of the present invention. Chemicals are small molecules of less than 500 daltons, while antigens are large molecules. As an example, CT is a protein antigen of about 85,000 daltons.

Accordingly, there is no motivation to combine the teachings of W0 95/17211 and U.S. Patent '499 and to modify the method disclosed in the cited references to obtain the claimed method with any reasonable expectation of success. Thus, the cited references do not render the claimed invention obvious.

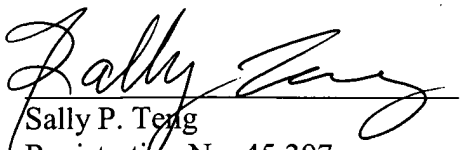
Conclusion

The foregoing amendments and remarks are being made to place the application in condition for allowance. Applicants respectfully request entry of the amendments, reconsideration, and the timely allowance of the pending claims. A favorable action is awaited. Should the Examiner find that an interview would be helpful to further prosecution of this application, they are invited to telephone the undersigned at their convenience.

If there are any additional fees due in connection with the filing of this response, please charge the fees to our Deposit Account No. 50-0310. If a fee is required for an extension of time

under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,
MORGAN, LEWIS & BOCKIUS LLP



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Nasal Vaccination, *Escherichia coli* Enterotoxin, and Bell's Palsy

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In 1997, a Swiss Company (Berna Biotech) received approval to market an inactivated influenza vaccine for parenteral administration that consists of the hemagglutinin and neuraminidase surface antigens of influenzavirus incorporated into liposomes. This virosome vaccine was subsequently used in the formulation of an influenzavirus vaccine for intranasal administration. To optimize both mucosal and systemic immune responses to the nasal vaccine, heat-labile *Escherichia coli* enterotoxin, one of the most powerful mucosal adjuvants known, was included in the formulation. The clinical studies showed that clinically significant antibody responses to influenzavirus were elicited by this vaccine, as evidenced in both nasopharyngeal secretions and serum, and that the vaccine was protective against influenza in both adults and children. Approval of the vaccine for distribution and use in Switzerland followed.

After Bell's palsy was identified in some recipients of this intranasal vaccine, however, it was withdrawn from the market. The report by Mutsch et al. in this issue of the *Journal* (pages 896–903) presents very strong evidence that the occurrence of Bell's palsy resulted from the use of the vaccine. The strong relation in the case–control study is supported by the case-series analysis that identified an increase in incidence, with a peak occurring between 31 and 60 days after vaccination and then a return to the base-line level of risk. Since the vaccine contained more than inactivated influenzavirus, it is important to consider the reasons for the increased risk of Bell's palsy. Nevertheless, the finding indicates a problem for nasal vaccination, which is an evolving approach to the prevention of influenza and other infectious diseases.

Is there cause for concern about the use of the current influenza vaccines? The results of the study indicate clearly that the parenteral use of inactivated influenza vaccine did not confer an increased risk of Bell's palsy. Thus, the current public health procedure of administering inactivated influenza vaccine parenterally for the prevention of influenza and its complications is not threatened by the finding. Is there cause for concern about the use of the new live attenuated vaccine (FluMist), which is given intranasally? Although some variation in the prevalence of Bell's palsy has been reported, it does not appear to occur in a seasonal pattern, and no relationship between its occurrence and influenza epidemics has been described. Since natural influenzavirus infection does not appear to be a precipitating event for Bell's palsy, there is no reason to suppose that an attenuated infection induced by a live vaccine such as the intranasal vaccine would confer an increased risk of this condition. Thus, there is little reason for concern about the continued use of the new live influenzavirus vaccine.

There is a need for improvement in the protection afforded by the current inactivated influenza vaccines, and intranasal vaccination is one possible alternative. Of greater concern than the development of alternative influenza vaccines, however, is the future of intranasal vaccination as an option for the prevention of infectious disease. Many consider the nasal route to be the most effective route for inducing both mucosal and systemic immunity to an infectious agent and believe that the exploitation of the advantages of this route of administration will be the basis for the next generation of vaccines. An awareness of these advantages for the administration of antigens (see Table) has led to repeated demonstrations in animals and humans of the potential value of intranasal vaccines for the prevention of at least 10 viral infections, 10 bacterial infections, and 2 parasitic infections. The requirement that a mucosal adjuvant be incorporated into the vaccine in order to elicit optimal responses has led to the demonstration of the value of about 10 different adjuvants. Thus, the effort involved in developing intranasal vaccines is extensive.

Elucidating the pathogenesis of the Bell's palsy that occurred after the administration of the Berna intranasal vaccine is a high priority for those involved in vaccine development. Was the increased risk attributable to the inactivated influenzavirus,

the *E. coli* enterotoxin, both of these components, the route of administration, or some other factor? Since parenteral administration of influenza vaccine did not confer a similar risk, inactivated influenzavirus was not the cause. The authors sought but did not identify another factor that might have had a contributing effect; therefore, the *E. coli* toxin, the combination of influenzavirus and the toxin, or the route of administration remain the most likely possibilities. Given that the respiratory tract of humans is challenged with antigens almost on a daily basis and that frequent viral and bacterial infections, carriage of bacterial antigens, and inert environmental antigens induce numerous host responses, including immune responses, it seems unlikely that the intranasal administration of inactivated influenzavirus would elicit a qualitatively or quantitatively unique response that would precipitate the characteristic inflammatory seventh-nerve response of Bell's palsy. There are data, however, suggesting that the *E. coli* enterotoxin in the vaccine may be the risk-inducing factor.

Cholera toxin and heat-labile *E. coli* enterotoxin are very potent mucosal adjuvants; the B subunit of these toxins binds to gangliosides on the cell surface, leading to internalization, and the A subunit is responsible for the activation of adenyl cyclase, elevated cyclic AMP levels, and the water and chloride secretion that leads to diarrhea during intestinal infection. Both the A and B subunits exert adjuvant effects through a variety of actions. After the intranasal administration of antigen in mice, both antigen and toxin were found in the olfactory nerve and the olfactory bulb for an extended period; antigen accumulation did not occur in the absence of toxin. In addition, the intranasal enterotoxin induced inflammatory responses in the olfactory sites and the meninges of mice. Thus, there is a reason to be concerned about neurotoxic effects of the intranasal administration of a vaccine containing an enterotoxin adjuvant.

Table. Advantages and Disadvantage of Intranasal Vaccinations.

Advantages
Ease of administration; no need for injections
Small dose needed; generally more effective than oral route
Leads to both mucosal and systemic immune responses
Immune responses occur at a pathogen-entry site
Induces responses at other mucosal sites (common system)
Some functional advantages of secretory IgA over standard IgA and IgG
Exhibits long-term memory
Disadvantage
Frequently requires adjuvant for optimal response

What intranasally induced responses led to Bell's palsy? The fact that the interval of maximal occurrence in the Mutsch study was 31 to 60 days after vaccination suggests that an induced response, rather than some direct toxic effect, led to the palsy. The prevailing notion is that most cases of Bell's palsy represent an autoimmune disorder or a reactivation of a latent herpesvirus infection. Both herpes simplex virus and varicella–zoster virus have been shown to be latent in a high proportion of seventh-nerve ganglia. The well-known Ramsay Hunt syndrome involves the reactivation of varicella–zoster virus, with a resulting seventh-nerve palsy and local cutaneous lesions. Herpes simplex virus is less well established as a cause of Bell's palsy, but the evidence of a causal relation is strong, and it is possible that a herpesvirus infection was reactivated in the patients in whom the palsy developed. Identification of the cause of Bell's palsy associated with the use of the intranasal *E. coli*–adjuvant influenza vaccine and other intranasal vaccines is needed to facilitate further development of intranasal vaccines, including vaccines for influenza.

From the Baylor College of Medicine, Houston.

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Improved immune responses to influenza vaccination in the elderly using an immunostimulant patch

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Abstract

The elderly have greater morbidity and mortality due to influenza, and respond poorly to influenza vaccination compared to younger adults. This study was designed to determine if the adjuvant heat-labile enterotoxin from *Escherichia coli* (LT), administered as an immunostimulant (IS) patch on the skin with influenza vaccination, improves influenza immune responses in the elderly. Three weeks following vaccination, hemagglutination inhibition (HAI) responses in LT IS patch recipients showed improvement over those of elderly receiving vaccine alone, as demonstrated by significance or trends in fold rise [A/Panama ($P = 0.004$), A/New Caledonia ($P = 0.09$)], seroconversion [A/New Caledonia (63% versus 40%, $P = 0.01$), A/Panama (54% versus 36%, $P = 0.08$)] and seroprotection [26%, 20% and 16% greater for the patch group for A/New Caledonia, A/Panama and B/Shandong strains, respectively]. The data suggest that an LT IS patch may further enhance influenza vaccine immune responses in the elderly.

Keywords: Elderly; Influenza; Immunostimulant patch; Adjuvant; LT

1. Introduction

Influenza vaccination is a critical weapon in the public health armamentarium for protection against the annual attack of influenza virus, most notably in the elderly where high rates of influenza-related death and morbidity occur [1,2]. The susceptibility of elderly to influenza complications is further exacerbated by other underlying illnesses, frailty, and possible deficits in non-specific host defense factors [3]. Enhancement of vaccine efficacy in the elderly is a fundamental unmet need for influenza vaccination, as well as for other vaccines that target the elderly. It is not widely appreciated that influenza vaccine response rates are far from satisfactory in the elderly, and can be as low as 17–35% depending on the vaccine strain and year [4,5]. The poor response to vaccination is common knowledge among influenza specialists and regulatory authorities. In Europe, where licensed vaccines are tested annually for efficacy, different standards of evaluation of immunogenicity are accepted by the European Agency for the Evaluation of Medicinal Products (EMEA) for subjects 18–60 years of age and for subjects older than 60 due to the low magnitude and response rate to influenza vaccination in the elderly [6]. The mechanism for decreased influenza vaccine efficacy in the elderly is not well understood, but is likely to be related to immune senescence, especially in T cells and antigen-presenting cells [7]. However, the immune deficit is clearly seen by current standard measure of flu responses to vaccination in the form of hemagglutination inhibition (HAI) antibody responses. The relationship between HAI antibody responses and the clinical efficacy of influenza vaccines is still not fully understood, however, an increase in HAI antibody titer has been used as a surrogate marker for evaluating influenza vaccines, and is considered the best practicable

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correlate of protection. For regulatory purposes, HAI titers are an integral part of influenza license applications, and the basis for the yearly approval of the seasonal formulation of influenza vaccines in Europe [6].

Adjuvants act as potent immune stimulators that may close the gap between the normal adult immune responses and those of the elderly. There are several adjuvants that are used in licensed vaccines including alum, cholera toxin B, MF59, and virosomes [8]. To date, two adjuvanted influenza vaccines that have been shown to enhance the influenza specific immune response are available in limited markets [9–11].

We have recently described a new adjuvant administration strategy that targets the human skin immune system using a patch [12,13,14]. The epidermis is equipped with a potent immune cell, the Langerhans cell (LC), that densely populates the most superficial layer of the living skin. The heat-labile toxin of *Escherichia coli* (LT) can be used as an efficient activator of resident LCs [15]. The simple, topical placement of an adjuvant-laden patch on the skin results in trafficking of activated Langerhans cells to the draining lymph nodes where immune responses are generated as a part of normal host defense [16,17]. The presence of adjuvant-activated LCs in the draining lymph node appears to have positive bystander effects on injected vaccines [12,17]. Specifically, in preclinical studies we have observed that application of an adjuvant patch (immunostimulating or IS patch) directly over, or in close proximity to, the site of injection augments the specific immune response to the injected vaccine [12,13]. To test the hypothesis that an IS patch could enhance the flu-specific immune response in the elderly, we applied an IS patch to subjects over 60 years of age receiving their annual influenza vaccine, and found that the addition of the adjuvant patch improved the influenza specific immune response in humans.

2. Materials and methods

This open-label study was conducted in 166 healthy male and female subjects who were recruited from Allschwil, Switzerland and the surrounding environs. Subjects were not considered if they had evidence of acute disease at the time of vaccination or any known immunodeficiency. Eligible subjects signed written informed consent prior to study enrollment. All study participants received a single, intramuscular dose of virosomal influenza vaccine, containing 45 μg hemagglutinin (HA) (0.5 ml of Inflexal V®, Berna Biotech Ltd., Bern, Switzerland) in the deltoid. The influenza vaccine contained HA of the influenza strains recommended by the WHO for the 2002–2003 season: A/New Caledonia/20/99(H1N1)-like; A/Moscow/10/99(H3N2)-like; and B/Hong Kong/330/2001-like virus. The 55 adults (ages 18–59) and first 55 elderly (>60 years) received only influenza vaccine. The final 56 elderly received both influenza vaccine, and 45 μg of LT in buffer on a patch placed approximately 5 cm distal to the vaccine injection site. Prior to application, the patch area was lightly abraded with EKG grade emery paper on skin wetted with 10% glycerol/70% alcohol to disrupt the stratum corneum. The adjuvant LT in buffer (Berna Biotech Ltd., Bern, Switzerland) was diluted with saline to yield a dose of 45 μg/150 μl solution. Each dose was applied to a gauze pad, and then covered with a Tegaderm overlay. Subjects wore the patch for 6 h, then the patch was removed and the arm rinsed.

Subjects were observed for 30 min post-vaccination to evaluate any acute reactions following vaccination and, in the case of patch recipients, assess patch adhesion. Subjects were given a diary card to record local and systemic reactions that occurred following immunization. Those who received influenza vaccine alone were specifically asked to report the following local and systemic reactions: induration >5 mm, redness >5 mm and ecchymosis >5 mm, malaise, shivering and fever (body temperature >38.0°). In addition to this list of solicited adverse events, patch recipients were asked to record any pain, itching or rash associated with the patch or tape overlay, as well as diarrhea.

Serum samples from days 0 and 21 were simultaneously tested for strain specific HAI by Retroscreen Virology, Ltd. Results were reported for A/New Caledonia/20/99, A/Panama/2007/99 (an A/Moscow/10/99-like strain) and B/Shandong/7/97 (a B/Hong Kong/330/2001-like strain). The humoral response to the three influenza vaccine strains was assessed by calculating the following: (1) geometric mean titers (GMT) before vaccination and 21 days after administration; (2) GMT fold ratio of day 21 post-vaccination titers to baseline titers; (3) seroconversion rate (percentage of subjects showing a ≥four-fold increase in HAI titers 21 days after vaccination compared to baseline and a titer of ≥1:40); and (4) seroprotection rate (percentage of subjects with a titer ≥1:40) before vaccination as well as 21 days after vaccination.

3. Results

3.1. Safety results

All adverse events reported during this trial were mild or moderate, and transient in nature. A mild, self-limited pruritic rash was reported in 13% ($n = 7$) of patch recipients. Nine percent ($n = 5$) of patch recipients reported mild diarrhea, but as described above these data were not collected for the vaccine alone groups, and thus may represent background diarrhea incidence not related to LT as adjuvant. The frequency and severity of all other local and systemic adverse events did not differ significantly for the different treatment groups.

3.2. Immunogenicity

The fold rise in the HAI titers against the three influenza strains was significantly greater in young adults compared to elderly subjects given vaccine alone, consistent with the rel-

Table 1
HAI geometric mean titer and fold rise by treatment group

Group	Influenza strain	Day 0 HAI (GMT)	Day 21 HAI (GMT)	Fold rise (95% CI)
Adults (18–59 years, $n = 55$)	A/New Caledonia	17	175	10.5 (6.4, 17.5)
	A/Panama	20	88	4.5 (3.1, 6.5)
	B/Shandong	6	27	4.5 (3.2, 6.2)
Elderly, no patch (≥60 years, $n = 55$)	A/New Caledonia	14	43	3.2 (2.2, 4.6)[a]
	A/Panama	39	92	2.3 (1.6, 3.3)[a]
	B/Shandong	8	17	2.2 (1.6, 3.2)[a]
Elderly, IS patch (≥60 years, $n = 56$)	A/New Caledonia	13	62	5.1 (3.4, 7.7)[a]
	A/Panama	20	103	5.1 (3.4, 7.5)[b]
	B/Shandong	6	18	3.0 (2.3, 4.0)

[a] Significant difference between elderly (±IS patch) and healthy adults ($P < 0.05$).
[b] Significant difference between elderly and elderly with IS patch ($P = 0.004$).

ative immune deficit seen elsewhere in the elderly, as shown in Table 1. The addition of the IS patch to the elderly raised the HAI fold rise to all three strains compared to the elderly without the patch, that reached significance in the A/Panama strain, with a trend in the A/New Caledonia strain ($P < 0.09$). Subjects in this study appeared to be relatively naïve to the B/Shandong strain and post-vaccination HAI titers in all groups were low. This is likely due to the replacement of the B strain in this year's vaccine, and is consistent with responses to a priming dose compared to the booster responses in the A strains. Additionally, there was no significant difference in the fold rise between young adults and elderly adults with a patch for A/Panama and B/Shandong, suggesting that the IS patch enhancement partially closed the gap between young and elder immune responsiveness.

Seroconversion, a four-fold rise in HAI antibody titers, has been used by convention to indicate a significant immune response to influenza vaccination. As shown in Fig. 1, young adults had 69%, 56%, and 61% seroconversion rates for A/New Caledonia, A/Panama and B/Shandong, respectively. These rates were significantly higher or showed a trend compared to corresponding seroconversion rates in the elderly without a patch (40% [$P = 0.005$], 36% [$P = 0.06$], and 38% [$P = 0.03$], respectively), a finding consistent with the relative

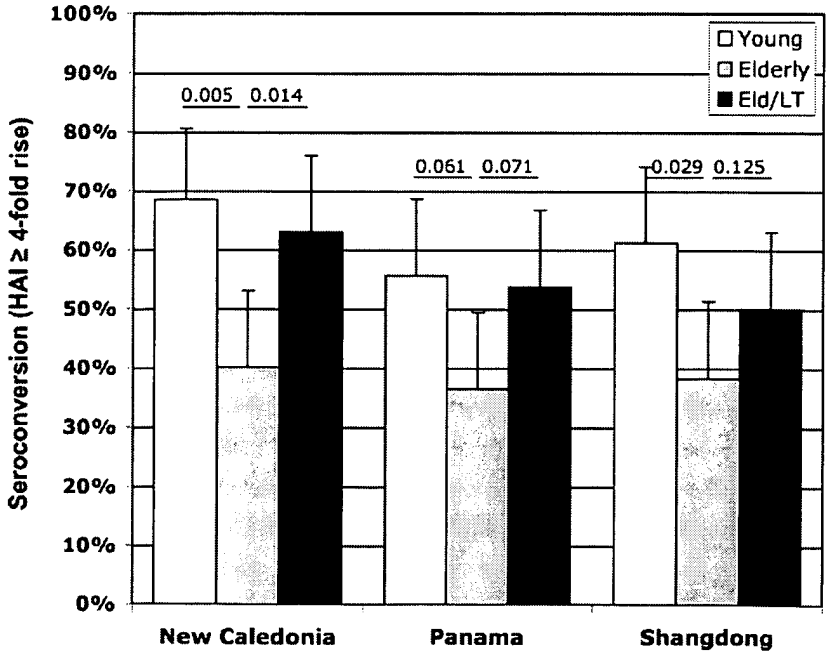

Fig. 1. Seroconversion rates at day 21 by treatment group for A/New Caledonia, A/Panama and B/Shandong. Young adults had 69%, 56%, and 61% seroconversion rates for A/New Caledonia, A/Panama and B/Shandong, respectively. These rates were significantly higher or showed a trend compared to corresponding seroconversion rates in the elderly without a patch (40% [$P = 0.005$], 36% [$P = 0.06$], and 38% [$P = 0.03$], respectively). The addition of an IS patch to the elderly improved these seroconversion rates to 63%, 54%, and 50%, respectively, reaching significance in the A/New Caledonia strain ($P = 0.01$) and a trend for A/Panama ($P = 0.08$). The seroconversion rates in elderly receiving the patch were not significantly different from those in healthy adults, and represent an absolute improvement of 23% (A/New Caledonia), 18% (A/Panama) and 12% (B/Shandong) in seroconversion of the elderly over those not receiving the patch.

Table 2
Seroprotection rates and percentage change by treatment group

Group	Influenza strain	Day 0 (%)	Day 21 (%)	Change (%)
Adults (18–59 years, $n = 55$)	A/New Caledonia	31	91	60
	A/Panama	29	89	60
	B/Shandong	2	46	44
Elderly, no patch (≥60 years, $n = 55$)	A/New Caledonia	29	58	29
	A/Panama	60	89	29
	B/Shandong	11	29	18
Elderly, IS patch (≥60 years, $n = 56$)	A/New Caledonia	19	73	55
	A/Panama	35	84	49
	B/Shandong	2	36	34

immune deficit in the aged. The addition of an IS patch to the elderly improved these seroconversion rates to 63%, 54%, and 50%, respectively, reaching significance in the A/New Caledonia strain ($P = 0.01$) and a trend for A/Panama ($P = 0.08$). The seroconversion rates in elderly receiving the patch were not significantly different from those in healthy adults, and represent an absolute improvement of 23% (A/New Caledonia), 18% (A/Panama) and 12% (B/Shandong) in seroconversion of the elderly over those not receiving the patch.

Seroprotection, the percentage of subjects achieving an HAI titer ≥1:40 post-vaccination, was achieved in most subjects for the A/New Caledonia and A/Panama strains, and lower levels were seen for the B/Shandong, reflecting the relative naïveté of subjects to this strain. The percent change in seroprotection rates following vaccination was highest for the healthy adults, followed by the group receiving an IS Patch (Table 2). Overall, the IS patch group improved the elderly seroprotection rates by 26%, 20%, and 16% compared to influenza vaccination alone for the A/New Caledonia, A/Panama and B/Shandong strains, respectively.

4. Discussion

Influenza exacts a disproportionate toll on the elderly, as evidenced by increased morbidity and mortality from associated pneumonia and other pulmonary and cardiac illness in this population [18]. The confluence of waning immune competence, poor immune responsiveness to vaccines, and co-morbidity with other chronic or infectious diseases renders the elderly vulnerable to influenza infection, and at increased risk of influenza-associated complications. Annual pandemics afflict the elderly, and result in increased hospital admissions and death. Current influenza vaccines have a major positive impact on mortality and morbidity, yet leave a significant percentage of elderly vaccine recipients with inadequate levels of influenza-specific antibodies [19,20]. This study shows that the addition of an IS patch to influenza immunization in the elderly leads to improved influenza-specific immune responses.

The skin immune system is a highly attractive target for vaccines, as it offers both an optimal target for vaccine delivery in the Langerhans cell and a safe method for delivery of strong adjuvants, such as LT [15,17,21]. The bacterial toxin LT, as well as other adjuvants, can have potent immune effects, but these agents often carry a risk of side effects that parallels their strength; however, the skin offers a well-circumscribed anatomical site that allows safe LT use. When placed on the skin, LT activates Langerhans cells that can be detected in the draining lymph nodes, but not in non-draining lymph nodes [12,17]. In the preclinical setting, the topical application of adjuvants such as LT consistently and significantly augments the immune response to vaccine antigens applied on the skin, as well as vaccines administered by alternate routes (intramuscular, oral [22], subcutaneous, intradermal). Specifically, preclinical studies have demonstrated that an LT patch, placed on the skin to target the same draining lymph nodes as an injected influenza vaccine, greatly enhances the influenza-specific immune responses.

In the clinical trial, a simple patch was added to an annual influenza vaccine product evaluation. Despite the fact that the study was not powered for significance, significant immune enhancement effects were seen. The relative immune deficit of the elderly compared to healthy adults was observed for all three influenza strains, yet by addition of the IS patch the gap was closed for two of the three vaccine strains (A/New Caledonia and A/Panama). As is often the case, the subjects were relatively naïve for the B strain and, as might be expected, less of an IS effect was seen.

This trial employed a simple gauze patch containing LT in buffer was used in this study. A more efficient, formulated patch has been designed to improve LT delivery and subsequent immunostimulation. To confirm the IS effect in the elderly several larger, powered, clinical trials are underway using this newer generation, formulated patch.

Overall, the elderly influenza immune enhancement observed in this study was consistent with preclinical studies using an IS patch [12,17]. The utility of an IS patch could extend beyond the application to annual influenza vaccination to include the potential for broader application to pandemic influenza vaccines, SARS, and other vaccine programs targeting the elderly that could benefit from further immune stimulation. The IS strategy may also be employed in regimens where dose sparing or shortened time to immunization would be of importance. In summary, the IS patch is a practical intervention that takes advantage of the highly competent

skin immune system, the benefits of the use of another potent adjuvant (LT), and the high safety profile of the non-invasive skin patch delivery of the latter, to enhance immune responses to influenza vaccination.

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We would like to thank Ms. Christina Villar from IOMAI Corporation for her oversight of the study, Ms. Franziska Maurer for her detailed monitoring of all data, and Dr. Monika Griot for facilitating the project. In addition, we would like to acknowledge the diligent work of Dr. Michael Seiberling, investigator, and Ms. Heidi Keller, clinical coordinator, both employees of Swiss Pharma Contract.

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ORIGINAL ARTICLE

Use of the Inactivated Intranasal Influenza Vaccine and the Risk of Bell's Palsy in Switzerland

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ABSTRACT

BACKGROUND

After the introduction of an inactivated intranasal influenza vaccine that was used only in Switzerland, 46 cases of Bell's palsy were reported.

METHODS

We conducted a matched case–control study and a case-series analysis. All primary care physicians, ear, nose, and throat specialists, and neurologists in German-speaking regions of Switzerland were requested to identify cases of Bell's palsy diagnosed in adults between October 1, 2000, and April 30, 2001. Each physician was invited to select three control patients for each patient with Bell's palsy, with matching according to age, date of the clinic visit, and physician. Vaccination information was provided by the physicians.

RESULTS

A total of 773 patients with Bell's palsy were identified. Of the 412 (53.3 percent) who could be evaluated, 250 (60.7 percent) were enrolled and matched with 722 control patients; the other 162 patients had no controls. In the case–control study, we found that 68 patients with Bell's palsy (27.2 percent) and 8 controls (1.1 percent) had received the intranasal vaccine ($P<0.001$). In contrast to parenteral vaccines, the intranasal vaccine significantly increased the risk of Bell's palsy (adjusted odds ratio, 84.0; 95 percent confidence interval, 20.1 to 351.9). Even according to conservative assumptions, the relative risk of Bell's palsy was estimated to be 19 times the risk in the controls, corresponding to 13 excess cases per 10,000 vaccinees within 1 to 91 days after vaccination. In the case-series analysis, the period of highest risk was 31 to 60 days after vaccination.

CONCLUSIONS

This study suggests a strong association between the inactivated intranasal influenza vaccine used in Switzerland and Bell's palsy. This vaccine is no longer in clinical use.

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INFLUENZA IS A LEADING CAUSE OF ILLness and death.[1] Annual vaccination is recommended for certain age groups and high-risk populations. However, the acceptance of parenteral influenza vaccines has frequently been unsatisfactory, in part because of the fear of injections.[2] Intranasal administration of influenza vaccines is an attractive alternative. It might also reduce the transmission of influenza more efficiently than parenteral administration by stimulating both mucosal and systemic immune responses.[2,3] To date, two trivalent intranasal influenza vaccines have been developed. An inactivated virosomal-subunit influenza vaccine licensed in Switzerland (Nasalflu, Berna Biotech) was available for the 2000–2001 influenza season; it contained *Escherichia coli* heat-labile toxin as a mucosal adjuvant. A different live attenuated, cold-adapted influenza vaccine was recently licensed in the United States.[4]

No serious adverse events were reported with the Swiss vaccine in the prelicensure trials conducted among 1218 volunteers during four winter seasons (1996 to 1999).[5-9] It was introduced to the Swiss market in October 2000 as the first licensed intranasal influenza vaccine in the world.

During the seven-month period beginning that month (i.e., October 2000 to April 2001), the Swiss Drug Monitoring Center and various University of Zurich institutions received 46 sentinel case reports of Bell's palsy among recipients of the vaccine, including 43 from German-speaking parts of Switzerland. The media reported these events in March 2001. Subsequently, Berna Biotech suspended distribution of the vaccine and invited our institutions at the University of Zurich to investigate whether the inactivated intranasal vaccine was associated with an increased risk of Bell's palsy. The Centers for Disease Control and Prevention joined the investigation in November 2001. The Swiss intranasal vaccine is now no longer in clinical use.

There has been no systematic survey of Bell's palsy in Switzerland. The reported incidence of Bell's palsy in other countries has ranged from 15 to 40 cases per 100,000 population per year.[10-16]

METHODS

STUDY DESIGN

We conducted a matched case–control study in which patients with Bell's palsy (case patients) and control patients were matched according to age, date of their clinic visit, and physician. Since about 74,000 doses of the intranasal vaccine, or more than 80 percent of the intranasal-vaccine supply, were distributed in the 19 German-speaking regions of Switzerland, this region was defined as the study area. To gain a better understanding of the timing of the risk for Bell's palsy after administration of the intranasal vaccine, we assessed the onset intervals (i.e., the intervals from the date of the first dose of the vaccine to the date of the first visit to a physician for Bell's palsy) in a larger series of patients, a method referred to as case-series analysis.

The study protocol was approved by the ethics committees of the regions involved in the study. All the patients gave written informed consent, except for a small minority with additional cases reported by physicians anonymously. The academic investigators had access to all the study data, took responsibility for designing and conducting the analysis, and had authority over the manuscript preparation and the decisions concerning publication.

SELECTION OF CASE PATIENTS AND CONTROLS

All 4891 primary care physicians, ear, nose, and throat specialists, and neurologists in the study area were invited twice to report cases of Bell's palsy first diagnosed between October 1, 2000, and April 30, 2001. The overall response rate was 81.1 percent. Subsequently, the physicians who had reported cases of Bell's palsy were asked to document the date of the visit and information pertinent to the study's inclusion and exclusion criteria and to select, from among their patients without Bell's palsy, three controls sequentially from their registration log. The controls were matched with the case patients according to age (within five years), date of the clinic visit (within four days), and physician. Trained study monitors contacted the physicians and reviewed the selection forms regularly to ensure consistency in the selection of controls. At this point, participating physicians had not been made aware of the exposure to be investigated (influenza vaccination). Thereafter, questionnaires requesting clinical information about the case patients and controls were sent together with informed-consent forms to both the physicians and their patients. The patients' questionnaires were used primarily for validation of the information provided by the physicians. When there was a discrepancy, both the patient and the physician were contacted for clarification.

Patients with Bell's palsy were eligible for the

study if they were at least 18 years of age and if they had complete or incomplete unilateral weakness of the facial muscles. The onset of weakness had to be sudden, and the progression of facial palsy had to reach a maximum within one week after the onset of the first symptoms. Exclusion criteria were head trauma within two months before enrollment and a history of brain tumor, cerebrovascular accident, ipsilateral ear disease or surgery, or the Guillain–Barré syndrome. Patients were not selected as controls if they met the same exclusion criteria or if they received a clinical diagnosis of influenza at the index visit. The exclusion of patients with influenza as controls was a measure taken in an effort to prevent an overrepresentation of patients with influenza as controls during the winter season. Physicians who were unwilling to select controls were still asked to document any cases.

EXPOSURE TO INFLUENZA VACCINES

Physicians were asked to document the dates of administration and the brand name and type of influenza vaccine (parenteral or intranasal) used during the study period. Other vaccine exposures during the study period and the preceding two months were also documented. Since in all 43 sentinel cases reported in the study area the onset of Bell's palsy occurred within 91 days after intranasal vaccination, we defined the period of 1 to 91 days as the post-exposure risk period.

For 10 exposed case patients and 1 exposed control, we were unable to calculate the onset interval because the date of vaccination was unknown. We decided to include them in the conditional logistic-regression analysis on the assumption that they had an onset interval of 1 to 91 days, because including them would provide a more conservative estimate of the adjusted odds ratio for Bell's palsy with use of the intranasal vaccine than would excluding them.

COVARIATES

Additional information, including potential risk factors for Bell's palsy,[11-21] was obtained from physicians and patients. Documented information at the time of enrollment included sex, nationality, pregnancy status, smoking status, current infectious diseases (upper respiratory infections, influenza, herpes simplex, other herpes infections, borreliosis, human immunodeficiency virus infection, and *Mycoplasma pneumoniae* infection), chronic diseases (diabetes, cardiovascular diseases, hypertension, asthma, allergies, and neoplasm), and a personal or family history of Bell's palsy.

STATISTICAL ANALYSIS

Matched Case–Control Study

The date of the first visit to a physician for Bell's palsy was used as the index date and was documented for all the case patients. The date of administration of the first dose of the intranasal vaccine was defined as the exposure date. Conditional logistic regression (SAS version 8, SAS Institute) was performed to estimate the risk of Bell's palsy during the period from 1 to 91 days after vaccination (the predefined risk period), with adjustment for the potential risk factors defined as covariates.

Case-Series Analysis

To determine which exposure periods after vaccination were associated with the highest risk of Bell's palsy, we performed a case-series analysis.[22,23] The case series included 412 case patients (250 from the case–control study and 162 from the group of case patients without controls). We compared the risk of Bell's palsy during various periods after intranasal or parenteral vaccination (1 to 30, 31 to 60, and 61 to 91 days, as compared with 92 or more days).

Risk Assessment after Adjustment for Biases

To assess the presence and the magnitude of selection bias, we compared the number of case patients who had been exposed to the intranasal vaccine during the case–control study with the number of the sentinel case patients and with the series of case patients for whom there were no matched controls. In addition, we estimated the relative and excess risks based on the risk in the adult population and the number of doses of the intranasal vaccine distributed in the study area.

RESULTS

STUDY POPULATION

A total of 1291 cases of Bell's palsy were reported during the seven-month period in the study area. Eighty-one (6.3 percent) represented duplicate cases, 187 (14.5 percent) involved patients who were not identifiable in the medical records, and 250 (19.4 percent) involved patients who were ineligible for the study because they were younger than 18 years of age (34 patients), had an onset of Bell's

palsy before or after the study period (101), did not have Bell's palsy (31), or met one or more exclusion criteria (84).

Of the remaining 773 patients with Bell's palsy, 361 (46.7 percent) were not enrolled because their providers declined to participate; the other 412 (53.3 percent) were enrolled. The diagnosis was confirmed by a specialist in 298 patients (72.3 percent) and by a primary care physician in 114 (27.7 percent). Of the 412 case patients, 250 (60.7 percent) were matched with 722 controls; the providers of the remaining 162 case patients declined to enroll controls.

CASE–CONTROL STUDY

Table 1 summarizes the characteristics of the case patients, controls, and all vaccinees. Sixty-eight of the 250 patients with Bell's palsy (27.2 percent) and 8 of the 722 controls (1.1 percent) had received the inactivated intranasal vaccine (P<0.001). Of the patients who had not received the intranasal vaccine, 27 of the 182 patients with Bell's palsy (14.8 percent) and 90 of the 714 controls (12.6 percent) had been immunized with parenteral influenza vaccine before the index date (Table 2).

Conditional logistic-regression analysis showed that the adjusted odds ratio for a diagnosis of Bell's palsy during the 91-day exposure period among recipients of intranasal vaccine, as compared with controls, was 84.0 (95 percent confidence interval, 20.1 to 351.9) (Table 2). In contrast, there was essentially no risk of Bell's palsy after receipt of the traditional, parenteral influenza vaccine (adjusted odds ratio, 1.1; 95 percent confidence interval, 0.6 to 2.0). The only risk factor for Bell's palsy other than receipt of the intranasal vaccine was a nation-

Table 1. Demographic Characteristics and Underlying Conditions of the Patients with Bell's Palsy and the Controls.

Variable	Case–Control Study			All Vaccine Recipients (Case Patients and Controls)		
	Case Patients (N=250)	Controls (N=722)	Additional Patients with Bell's Palsy (N=162)	Intranasal Vaccine (N=99)	Parenteral Vaccine (N=130)	Crude Odds Ratio (95% CI)*
Demographic characteristic						
Age — yr						
Mean	49.9	50.3	49.7	50.0	54.8	
Median	49.7	50.3	48.1	49.0	57.0	
Sex — no. (%)						
Female	136 (54.4)	389 (53.9)	76 (46.9)	60 (60.6)	73 (56.2)	1.0
Male	114 (45.6)	328 (45.4)	85 (52.5)	38 (38.4)	57 (43.8)	0.8 (0.5–1.4)
Unknown	0	5 (0.7)	1 (0.6)	1 (1.0)	0	
Nationality — no. (%)						
German-speaking countries	203 (81.2)	569 (78.8)	129 (79.6)	92 (92.9)	114 (87.7)	1.0
Other countries	21 (8.4)	54 (7.5)	30 (18.5)	1 (1.0)	6 (4.6)	0.2 (0–1.3)
Unknown	26 (10.4)	99 (13.7)	3 (1.9)	6 (6.1)	10 (7.7)	0.7 (0.3–2.1)
Relevant diagnosis or intervention — no. (%)						
Type 2 diabetes	21 (8.4)	51 (7.0)	17 (10.5)	6 (6.1)	32 (24.6)	0.2 (0.1–0.5)
Hypertension	61 (24.4)	173 (24.0)	40 (24.7)	28 (28.3)	67 (51.5)	0.4 (0.2–0.7)
Asthma	6 (2.4)	41 (5.7)	6 (3.7)	7 (7.1)	11 (8.5)	0.8 (0.3–2.4)
Infectious disease	47 (18.8)	205 (28.4)	32 (19.8)	21 (21.2)	45 (34.6)	0.5 (0.3–1.0)
Upper respiratory infection	35 (14.0)	123 (17.0)	21 (13.0)	13 (13.1)	29 (22.3)	0.5 (0.1–1.1)
Other vaccination	5 (2.0)	27 (3.7)	2 (1.2)	3 (3.0)	4 (3.1)	1.0 (0.2–5.4)

* The crude odds ratio was calculated to assess differences between the intranasally and the parenterally vaccinated patients. CI denotes confidence interval.

ality within southern Europe (adjusted odds ratio, 2.4; 95 percent confidence interval, 1.3 to 4.7). In addition, controls were more likely than case patients to have an infectious disease, although the difference was barely significant (adjusted odds ratio, 0.47; 95 percent confidence interval, 0.23 to 0.98).

Table 2. Risk of Bell's Palsy among Participants in the Case–Control Study.*

Vaccine and Bell's Palsy Onset Interval	Case Patients (N=250)	Controls (N=722)	Adjusted Odds Ratio (95% CI)
	no. (%)		
Intranasal vaccine†			
Onset interval ≤91 days	63 (25.2)	7 (1.0)	84.0 (20.1–351.9)
Onset interval >91 days	5 (2.0)	1 (0.1)	
Parenteral vaccine			
Onset interval ≤91 days	10 (4.0)	41 (5.7)	1.1 (0.6–2.0)
Onset interval >91 days	17 (6.8)	49 (6.8)	

* The date of vaccination (the date of the first dose in the case of intranasal vaccine) and the date of the first visit to a physician for Bell's palsy (the index date) were used to determine the onset interval. This information was obtained from the medical records. Case patients and controls were matched according to age, date of the clinic visit, and physician. The odds ratios for Bell's palsy were estimated by conditional logistic-regression analysis. CI denotes confidence interval.

† For 10 case patients and 1 control, the onset interval was unknown.

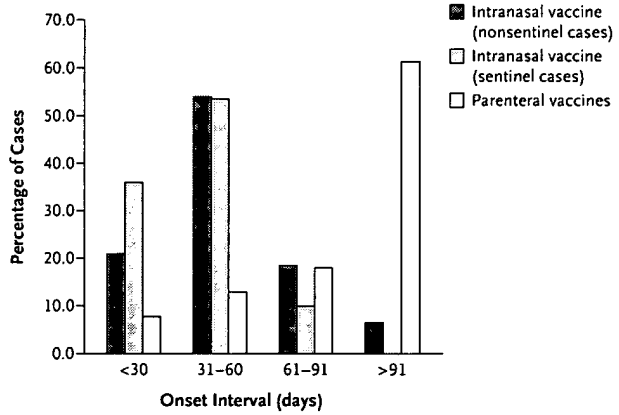

Figure 1. Onset Intervals among the Patients with Bell's Palsy.

Among nonsentinel cases of Bell's palsy in which the intranasal vaccine had been administered, the onset interval was defined as the period from the first dose of the intranasal vaccine to the first visit to a physician for Bell's palsy; among sentinel cases, the onset interval was defined as the period from the first dose of the intranasal vaccine to the onset of Bell's palsy. Among cases of Bell's palsy in which a parenteral vaccine had been administered, the onset interval was defined as the period from vaccination to the first visit to a physician for Bell's palsy.

CASE-SERIES ANALYSIS AND RISK PERIODS

In our case-series analysis of 412 patients, we identified 91 intranasally vaccinated patients with Bell's palsy, 81 of whom had adequate data for calculating onset intervals. Forty-four of these 81 patients (54.3 percent) first visited a physician because of symptoms 31 to 60 days after intranasal vaccination (relative incidence of Bell's palsy after intranasal vaccination, 35.6; 95 percent confidence interval, 14.1 to 89.8), 17 (21.0 percent) had an onset interval of 1 to 30 days (relative incidence, 14.0; 95 percent confidence interval, 5.2 to 37.9), 15 (18.5 percent) had an onset interval of 61 to 91 days (relative incidence, 11.8; 95 percent confidence interval, 4.3 to 32.3), and 5 (6.2 percent) had an onset interval exceeding 91 days (Fig. 1). The onset intervals in sentinel cases had a similar distribution, although in more than one third of the sentinel cases the onset interval was 1 to 30 days. In contrast, 61.5 percent of parenterally vaccinated case patients had an onset interval of more than 91 days. Table 3 provides data on the neurologic outcome in these patients.

RISK ASSESSMENT AFTER ADJUSTMENT FOR BIASES

We were concerned that in our case–control study we may have overestimated the risk of Bell's palsy after intranasal vaccination, for the following two reasons. First, sentinel cases had a higher probability of being included in the case–control study (22 of 43 cases [51.2 percent]) than nonsentinel cases (228 of 730 [31.2 percent]) (Table 4). Second, the proportion of patients with Bell's palsy who had been exposed to the vaccine was 27.2 percent (68 of 250 patients) in the case–control study but only 14.2 percent (23 of 162 patients) in the series of case patients without controls (Table 4). We used two approaches to estimate the total number of exposed case patients in the entire group of 773 patients with Bell's palsy in order to adjust for this likely overestimation.

Approach 1

We assumed that nonsentinel cases of Bell's palsy with vaccine exposure had the same probability of being included in either the case–control study or the case series as did sentinel cases. According to Table 4, this assumption forces the following equation: $(22+5)\div 43=(46+18)\div(64+x)$, which is solved when $x=38$ and which yields an estimate of 145 total (sentinel and nonsentinel) cases with vaccine exposure.

Approach 2

The proportion of cases with vaccine exposure was higher in the case–control study than in the case series, with an odds ratio of 2.25 (27.2 percent vs. 14.2 percent). We assumed that the odds of exposure in the case series was also 2.25 times the odds of exposure in the remaining, unreported cases. This assumption forces the following equation: $[0.142 \div (1-0.142)] \div [(16+x) \div (361-[16+x])] = 2.25$, which is solved when $x=9$ and which yields an estimate of 116 total exposed cases (Table 4).

The more conservative estimate of 116 cases of Bell's palsy with vaccine exposure, as calculated with the second approach, yields an overall risk associated with exposure of 15.0 percent (116 of 773 cases overall). Thus, among 250 cases in the case–control study, one would have expected 38 cases with exposure, rather than the observed 68. This suggests that the adjusted odds ratio in the case–control study may have been overestimated by a factor of $(0.272 \div [1-0.272]) \div (0.150 \div [1-0.150])$, or 2.1. When we accounted for the overestimation, the estimate of the adjusted odds ratio was reduced to 40.0 ($84.0 \div 2.1$).

We identified 107 cases of Bell's palsy with vaccine exposure from all sources of information (Table 4). We assumed that they constituted all the exposed cases (and that there were no additional instances of exposure among the 361 nonparticipating patients with Bell's palsy). We also had information on the size of the adult population in the study area (4.056 million) and on the doses of intranasal vaccine that were distributed for 74,000 vaccinees. The estimated 666 cases of Bell's palsy without exposure (773 cases overall, minus 107 cases with exposure) over a seven-month period in a population of 3.982 million adults (4.056 million overall, minus 0.074 million vaccinees) yields a rate of 29 cases per 100,000 population per year.

On the basis of the available onset intervals among the cases of Bell's palsy with vaccine exposure, we estimated that in 93.8 percent of these cases the palsy developed 1 to 91 days after vaccination. Combining this result with the minimum number of 107 for all cases of Bell's palsy with exposure would yield 100 as the number of intranasally vaccinated patients in whom the palsy developed within 1 to 91 days. This would yield an estimated rate of 541 cases of Bell's palsy per 100,000 vaccinees per year, with a relative risk of 19 for the three months after intranasal vaccination and a corresponding excess risk of 13 cases per 10,000 vaccinees.

DISCUSSION

We found that the intranasal influenza vaccine used in Switzerland during the 2000–2001 influenza season greatly increased the risk of Bell's palsy among vaccinees. The association was strong, temporal, and specific. The risk was highest during the second month after intranasal vaccination. In contrast, no significant risk of Bell's palsy was found to be associated with the parenteral influenza vaccines.

We faced substantial challenges in the process of collecting data. Because of strict medical-privacy standards in Switzerland, few physicians would allow us to review the medical records on site or off site, in the form of photocopies. The time required for physicians to enroll both cases and controls became a formidable burden. Consequently, we were able to enroll only about one third of the identified cases in the case–control study. Our data suggested that patients with Bell's palsy who had received the intranasal vaccine were overrepresented in the case–control study, probably because of the regional media coverage of this problem. Furthermore, the exact magnitude of the risk associated with use of the intranasal vaccine was difficult to quantify because of the small number of controls who had received it.

All these factors raised concern about the ability of the case–control study to provide unbiased results. However, even after we adjusted for possible biases, our risk assessment provided strong evidence supporting our findings. Even with the most conservative assumption — that the 107 documented cases with exposure were the only such cases — the association of Bell's palsy and intranasal vaccination was still overwhelming, with a relative risk of at least 19 and an excess risk of 13 cases per 10,000 vaccinees. The population-based risk esti-

Table 3. Outcome of Patients with Bell's Palsy.*

Cranial-Nerve Function†	Intranasal Vaccine (N=91)	Parenteral Vaccine (N=40)	No Vaccine (N=281)
		number (percent)	
Complete (100%)	73 (80.2)	27 (67.5)	180 (64.1)
Incomplete	15 (16.5)	7 (17.5)	62 (22.1)
≥95%	7 (7.7)	4 (10.0)	18 (6.4)
<95%	8 (8.8)	3 (7.5)	44 (15.7)
Unknown	3 (3.3)	6 (15.0)	39 (13.9)

* Because of rounding, not all percentages total 100.

† Cranial-nerve function (the function of the seventh cranial nerve) was documented by the patients' physicians.

Table 4. Distribution of Cases of Bell's Palsy According to the Data-Collection Process and Exposure to the Intranasal Vaccine.*

Case Patients	Cases with Vaccine Exposure		Cases without Vaccine Exposure	All Cases	Percentage of Cases with Vaccine Exposure
	Sentinel	Nonsentinel			
			number		
Enrolled in case–control study†	22	46	182	250	27.2
Enrolled without controls‡	5	18	139	162	14.2
Not enrolled	16	x	361−[16+x]	361	≥4.4
Total	43	64+x	321+[361−(16+x)]	773	

* Values for the variable x were found in each of two methods used to evaluate the probable overestimation of the risk of Bell's palsy after intranasal vaccination.
† These case patients were enrolled in both the case–control study and the case-series analysis.
‡ These case patients were enrolled only in the case-series analysis because controls were not made available by the participating physicians.

mate for the cases of Bell's palsy without exposure was within the ranges reported in the literature.[10-16] The relatively high risk of Bell's palsy among southern Europeans has been reported previously.[24,25] We cannot explain the finding that controls were more likely than case patients to have infectious diseases, since this variable included multiple diseases, each of which affected only a small number of patients.

The clinical features of the Bell's palsy affecting the patients in this study were consistent with those described in the literature.[21] About one in five patients in all the groups had an incomplete recovery; loss to follow-up was more common among parenterally vaccinated or unvaccinated patients than among intranasally vaccinated patients.

The causes and pathogenesis of Bell's palsy remain unclear. Herpes simplex has been suspected,[26-28] but autoimmune processes have also been considered.[21] The results of studies in animals have raised concern that the adjuvant *Escherichia coli* heat-labile toxin may be an inflammatory mediator,[3,29] but preclinical research on its toxicologic characteristics and biologic distribution did not support this idea,[30] and neurologic toxicity of this substance in humans has never been described. Further studies are needed to investigate the pathogenesis of Bell's palsy after use of the inactivated intranasal influenza vaccine. Adverse neurologic events, such as Bell's palsy, should be monitored for at least 60 days when any intranasal influenza vaccine is tested.

In the prelicensure trials of the intranasal influenza vaccine, no cases of Bell's palsy were reported among 1218 recipients (Spyr C, Berna Biotech: personal communication). However, 46 cases of Bell's palsy were reported shortly after licensure. Historically, the sample sizes for even the largest phase 3 trials before licensure have been based on efficacy, not on safety considerations. Unfortunately, as with the experience with the rotavirus vaccine and intussusception,[31] the risk of Bell's palsy found after licensure of the intranasal influenza vaccine could not have been detected beforehand, given the sample size in the prelicensure trials. These experiences may reinforce the arguments both for larger prelicensure safety trials[32] and for enhanced postlicensure surveillance.[33]

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Functional Histology

A TEXT AND COLOUR ATLAS

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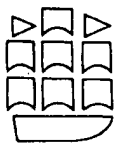

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4. Epithelial tissues

Introduction

The epithelia are a diverse group of tissues which, with rare exceptions, line all body surfaces, cavities and tubes. Epithelia thus function as interfaces between biological compartments. Epithelial interfaces are involved in a wide range of activities such as absorption, secretion and protection and all these major functions may be exhibited at a single epithelial surface. For example, the epithelial lining of the small intestine is primarily involved in absorption of the products of digestion, but the epithelium also protects itself from noxious intestinal contents by the secretion of a surface coating of mucus.

Surface epithelia consist of one or more layers of cells separated by a minute quantity of intercellular material which may represent the fused glycocalyces of adjacent cells (see Chapter 1); epithelial cells are closely bound to one another by a variety of specialisations of the cell membrane. All epithelia are supported by a *basement membrane* of variable thickness. Basement membranes separate epithelia from underlying connective tissues and are never penetrated by blood vessels; epithelia are thus dependent on the diffusion of oxygen and metabolites from underlying tissues. Basement membranes consist of a condensation of glycoprotein ground substance reinforced by reticular fibres which merge with those of the underlying connective tissue; both epithelial and connective tissue cells are thought to participate in the formation of basement membranes.

Epithelia are classified according to three morphological characteristics:

(i) The number of cell layers: a single layer of epithelial cells is termed *simple epithelium*, whereas epithelia composed of more than one layer are termed *stratified epithelia*.

(ii) The shape of the component cells when seen in sections taken at right angles to the epithelial surface: in stratified epithelia the shape of the outermost layer of cells determines the descriptive classification. Cellular outlines are often difficult to distinguish, but the shape of epithelial cells is usually reflected in the shape of their nuclei.

(iii) The presence of surface specialisations such as cilia and keratin: an example is the epithelial surface of skin which is classified as 'stratified squamous keratinising epithelium' since it consists of many layers of cells, the surface cells of which are flattened (squamous) in shape and covered by an outer layer of the proteinaceous material, keratin (see Fig. 4.14).

Epithelia may be derived from ectoderm, mesoderm or endoderm although in the past it was thought that true epithelia were only of ectodermal or endodermal origin; two types of epithelia derived from mesoderm, the lining of blood and lymphatic vessels and the linings of the serous body cavities, were not considered to be epithelia and were termed *endothelium* and *mesothelium* respectively. By both morphological and functional criteria, such distinction has little practical value, nevertheless, the terms endothelium and mesothelium are still used to describe these types of epithelium.

Epithelium which is primarily involved in secretion is often arranged into structures called *glands*. Glands are merely invaginations of epithelial surfaces which are formed during embryonic development by proliferation of epithelium into the underlying connective tissues. Those glands which maintain their continuity with the epithelial surface via a duct are called *exocrine glands* and secrete on to the free surface. In some cases, the duct degenerates during development to leave isolated islands of epithelial secretory tissue deep within other tissues. These glands, known as *endocrine* or *ductless glands*, secrete directly into the bloodstream and their secretions are known as hormones (see Chapter 14); in addition, some endocrine glands develop by migration of epithelial cells into connective tissues, without the formation of a duct.

Simple epithelia

Simple epithelia are defined as surface epithelia consisting of a single layer of cells. Simple epithelia are almost always found on absorptive or secretory surfaces; they provide little protection against mechanical abrasion and thus are almost never found on surfaces subject to such stresses. The cells comprising simple epithelia range in shape from extremely flattened to tall columnar, depending on their function. For example, flattened simple epithelia present little barrier to passive diffusion and are therefore found in sites such as the lung alveoli and the lining of blood vessels. In contrast, highly active epithelial cells, such as the cells lining the small intestine, are generally tall since they must accommodate the appropriate organelles. Simple epithelia may exhibit a variety of surface specialisations, such as microvilli and cilia, which facilitate their specific surface functions.

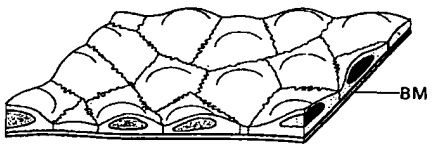

Fig. 4.1 Simple squamous epithelium

Simple squamous epithelium is composed of flattened, irregularly-shaped cells forming a continuous surface which is often referred to as *pavemented epithelium*. Like all epithelia, this delicate lining is supported by an underlying basement membrane **BM**.

Simple squamous epithelium is often found lining surfaces involved in passive transport of either gases, such as in the lungs, or fluids, such as the walls of blood capillaries. Simple squamous epithelium also forms a delicate lining to the pleural, pericardial and peritoneal cavities where it permits passage of tissue fluid into and out of these cavities; the simple squamous epithelium of these sites is traditionally known as mesothelium.

Fig. 4.2 Simple squamous epithelium

(H & E × 800)

This micrograph of a small blood vessel illustrates the typical appearance of simple squamous epithelium in section; the epithelial lining cells **E** (known as endothelium in the circulatory system) are so flattened that they can only be recognised by their nuclei which bulge into the vessel lumen. The supporting basement membrane is thin and, in haematoxylin and eosin stained preparations, has similar staining properties to the endothelial cell cytoplasm; hence the basement membrane cannot be seen in this micrograph.

Fig. 4.3 Simple squamous epithelium

(Spread preparation: silver method × 320)

In this preparation, the mesothelial lining of the peritoneal cavity has been stripped from the underlying connective tissues and spread onto a slide thus permitting a surface view of simple squamous epithelium. The intercellular substance has been stained with silver thereby outlining the closely interdigitating cell boundaries; the nuclei **N** have been stained with the dye, neutral red.

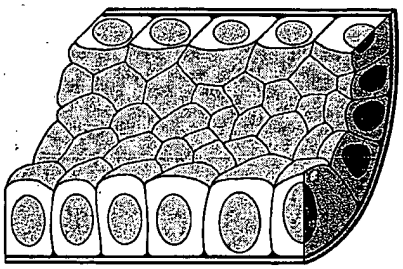

Fig. 4.4 Simple cuboidal epithelium

Simple cuboidal epithelium represents an intermediate form between simple squamous and simple columnar epithelia; the distinction between tall cuboidal and low columnar is often arbitrary and is of descriptive value only. In section perpendicular to the basement membrane, the epithelial cells appear square, leading to its traditional description as cuboidal epithelium; on surface view, however, the cells are actually polygonal in shape.

Simple cuboidal epithelium usually lines small ducts and tubules which may have excretory, secretory or absorptive functions; examples are the small collecting ducts of the kidney, salivary glands and pancreas.

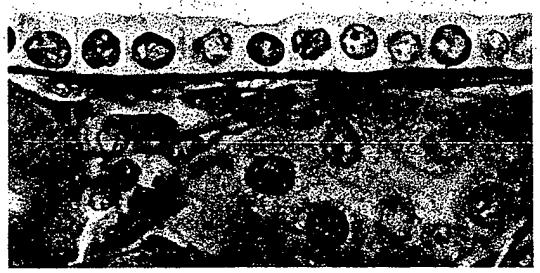

Fig. 4.5 Simple cuboidal epithelium

(Azan × 800)

This micrograph of the cells lining a small collecting tubule in the kidney shows simple cuboidal epithelium in section. Although the boundaries between individual cells are indistinct, the nuclear shape provides an approximate indication of the cell size and shape. The underlying basement membrane appears as a prominent blue line with this staining method.

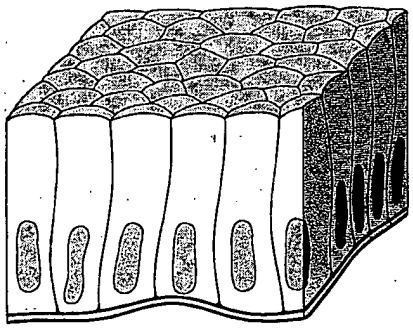

Fig. 4.6 Simple columnar epithelium

Simple columnar epithelium is similar to simple cuboidal epithelium except that the cells are taller and appear columnar in sections at right angles to the basement membrane. The height of the cells may vary from low to tall columnar depending on the site and/or degree of functional activity. The nuclei are elongated and may be located towards the base, the centre or occasionally the apex of the cytoplasm. When the nucleus is eccentrically placed, the cell is said to exhibit *polarity* which represents some internal compartmentation of the cytoplasm related to the specific function of the cell. Simple columnar epithelium is most often found on highly absorptive surfaces such as in the small intestine, although it may constitute the lining of highly secretory surfaces such as that of the stomach.

Fig. 4.7 Simple columnar epithelium

(H & E × 800)

This example of simple columnar epithelium is unusually tall and is taken from the lining of the gall bladder where it has the function of absorbing water, thus concentrating bile. The luminal plasma membranes of highly absorptive epithelial cells are often arranged into numerous, minute, finger-like projections called *microvilli* which greatly increase the surface area of the absorptive interface. Microvilli are usually too small to be resolved individually by light microscopy although they may collectively give the appearance of a *striated* or *brush border* at the luminal surface.

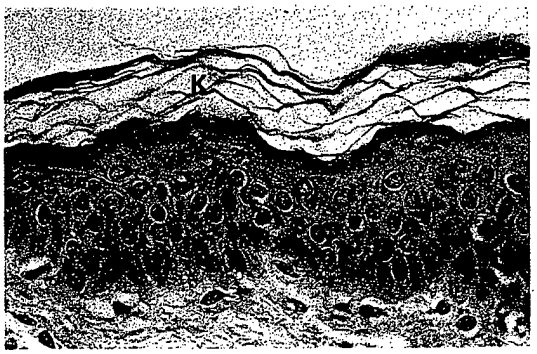

Fig. 4.14 Stratified squamous keratinising epithelium

(H & E × 320)

This specialised form of stratified squamous epithelium constitutes the epithelial surface of the skin and is adapted to withstand the constant abrasion and desiccation to which the body surface is exposed. During maturation, the epithelial cells undergo a process called *keratinisation* resulting in the formation of a tough, non-cellular surface layer consisting of the protein, *keratin* **K**, and the remnants of degenerate epithelial cells. Keratinisation may be induced in normally non-keratinising stratified squamous epithelium such as that of the oral cavity when exposed to excessive abrasion or desiccation.

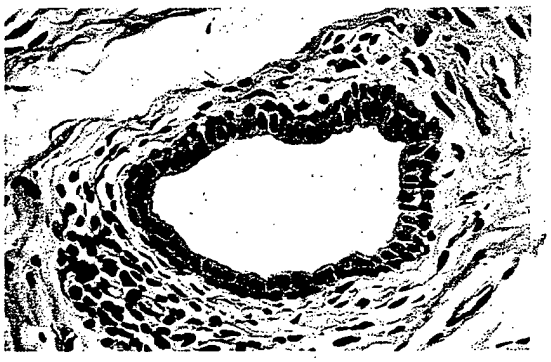

Fig. 4.15 Stratified cuboidal epithelium

(H & E × 320)

Stratified cuboidal epithelium is a thin, stratified epithelium which usually consists of only two or three layers of cuboidal or low columnar cells. This type of epithelium is usually confined to the lining of the larger excretory ducts of exocrine glands such as the salivary glands (as shown in this micrograph), the pancreas and sweat glands. Stratified cuboidal epithelium is probably not involved in significant absorptive or secretory activity but merely provides a more robust lining than would be afforded by a simple epithelium.

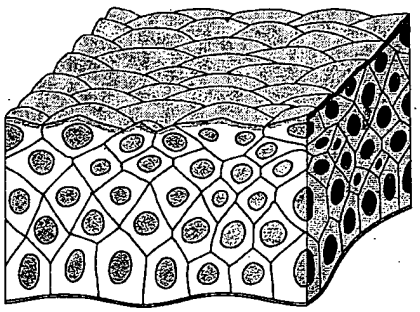

Fig. 4.16 Transitional epithelium

Transitional epithelium is a form of stratified epithelium almost exclusively confined to the urinary tract in mammals where it is highly specialised to accommodate a great degree of stretch and to withstand the toxicity of urine. This epithelial type is so named because it has some features which are intermediate between stratified cuboidal and stratified squamous epithelium. In the relaxed state, transitional epithelium appears to be about four to five cell layers thick; the basal cells are roughly cuboidal, the intermediate cells are polygonal and the surface cells are large and rounded and may contain two nuclei. In the stretched state, transitional epithelium often appears only two or three cells thick and the intermediate and surface layers are extremely flattened.

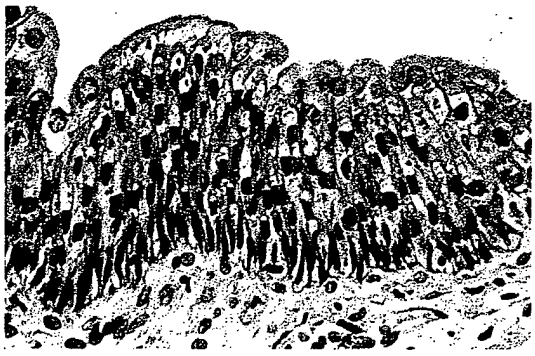

Fig. 4.17 Transitional epithelium

(H & E × 320)

This micrograph shows the appearance of transitional epithelium from the lining of a relaxed bladder. The shape and apparent size of the basal and intermediate cells vary considerably depending on the degree of distension, but the cells of the surface layer usually retain several characteristic features. Firstly, the surface cells are large and pale-stained and present a scalloped surface outline. Secondly, the luminal surface of the cells appears thickened and more densely stained. Thirdly, the nuclei of the surface cells are large and round, and often exhibit prominent nucleoli; some surface cells are binucleate.

8. Skin

Introduction

The skin, or integument, forms the continuous external surface of the body and in different regions of the body varies in thickness, colour and the presence of hairs, glands and nails. Despite these variations, which reflect different functional demands, all types of skin have the same basic structure. The external surface of skin consists of a keratinised squamous epithelium called the *epidermis*. The epidermis is supported and nourished by a thick underlying layer of dense, fibro-elastic connective tissue called the *dermis* which is highly vascular and contains many sensory receptors. The dermis is attached to underlying tissues by a layer of loose connective tissue called the *hypodermis* or *subcutaneous layer* which contains variable amounts of adipose tissue. Hair follicles, sweat glands, sebaceous glands and nails are epithelial structures termed *epidermal appendages* since they originate during embryological development from downgrowths of epidermal epithelium into the dermis and hypodermis.

The skin is the largest organ of the body, constituting almost one sixth of the total body weight; it has four major functions:

(i) Protection: the skin provides protection against ultraviolet light and mechanical, chemical and thermal insults; its relatively impermeable surface prevents excessive dehydration and acts as a physical barrier to invasion by micro-organisms.

(ii) Sensation: the skin is the largest sensory organ in the body and contains a variety of receptors for touch, pressure, pain and temperature.

(iii) Thermoregulation: in man, skin is a major organ of thermoregulation. The body is insulated against heat loss by the presence of hairs and subcutaneous adipose tissue. Heat loss is facilitated by evaporation of sweat from the skin surface and increased blood flow through the rich vascular network of the dermis.

(iv) Metabolic functions: subcutaneous adipose tissue constitutes a major store of energy, mainly in the form of triglycerides. Vitamin D is synthesised in the epidermis and supplements that derived from dietary sources.

Fig. 8.1 Skin (fingertip)

(Masson's trichrome × 8)

The general structure of skin is illustrated in this preparation of thick skin from the fingertip. The epidermis **E** consists of a stratified squamous keratinising epithelium which, in this site, has an extremely thick keratinised surface layer. A prominent feature of the skin of the fingertips, palms and soles of the feet is a pattern of surface ridges formed by the epidermis; this pattern is unique to each individual.

The epidermis is supported by the dermis **D**, a layer of dense fibro-elastic tissue, the fibres of which are stained green in this preparation. The dermis merges with the loose connective tissue of the hypodermis **H** which consists largely of adipose tissue; in this site, adipose tissue acts as a soft, shock-absorbing layer. Numerous sweat glands **S** are located in the dermis and hypodermis and discharge their secretions on to the skin surface via long excretory ducts **Dt**. Pressure receptors, Pacinian corpuscles **Pc** (see Fig. 7.35) are located deep in the dermis and are a prominent feature of fingertip skin.

The junction between the epidermis and dermis is characterised by downward folds of the epidermis called *epidermal ridges* which interdigitate with upward projections of the dermis called *dermal papillae*. This arrangement enhances the adhesion of the epidermis to the dermis and is accentuated in skin subject to considerable frictional forces.

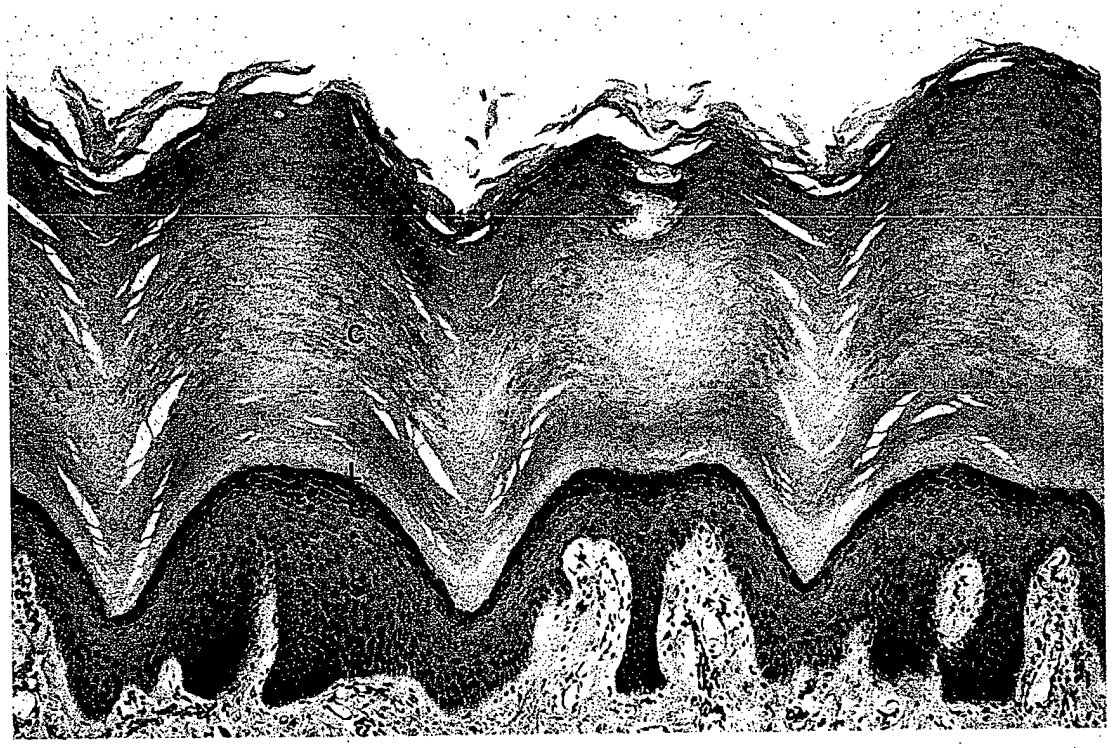

Fig. 8.2 Epidermis (fingertip)

(H & E × 104)

This section of thick skin, taken from the same specimen as Fig. 8.1, demonstrates the general features of the epidermis. Cells produced by mitosis in the germinal layer adjacent to the dermis undergo maturational changes concerned with the production of keratin. The outer keratinised layer is shed continuously and is replaced by the progressive movement and maturation of cells from the germinal layer. The rate of mitosis in the germinal layer generally equals the rate of desquamation of keratin from the outer surface.

The phases of this dynamic process are represented in five morphological layers:

(i) The stratum germinativum or stratum basale B is the germinal layer of the epidermis.

(ii) The stratum spinosum or prickle cell layer S, so named for the 'prickly' appearance of the cells at high magnification (see Fig. 8.4), contains cells which are in the process of growth and early keratin synthesis.

(iii) The stratum granulosum or granular layer G is characterised by the presence within the cells of granules which contribute to the process of keratinisation.

(iv) The stratum lucidum L is only present in extremely thick skin, and appears as a homogeneous layer between the stratum granulosum and the keratinised layer.

(v) The stratum corneum or cornified layer C consists of flattened, fused cell remnants composed mainly of the fibrous protein, keratin.

The process of maturation of a basal cell through to desquamation takes approximately 27 days in man.

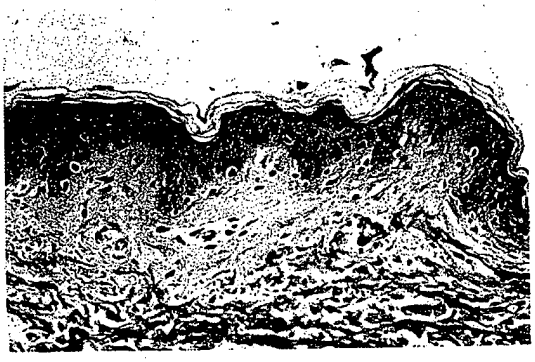

Fig. 8.3 Epidermis (abdomen)

(H & E × 128)

In this preparation of thin skin from the abdomen, the individual cellular layers are more difficult to discern and a stratum lucidum is not present. In comparison with thick skin, the stratum corneum is much reduced in thickness and the combined thickness of the other layers is reduced to a lesser extent.

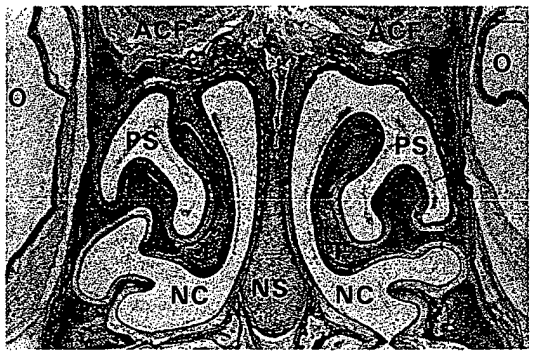

Fig. 11.1 Nasal cavity

(Kitten: coronal section: H & E|Alcian blue × 8)

The first part of the upper respiratory tract, the nose, is subdivided into two nasal cavities **NC** by the cartilaginous nasal septum **NS**; cartilage is stained blue in this preparation. The nasal cavities and paranasal sinuses **PS** are lined by respiratory mucosa, the major function of which is to filter particulate matter and to adjust the temperature and humidity of inspired air. These functions are enhanced by a large surface area provided by the turbinate system of bones **T** which project into the nasal cavities. Part of the nasal mucosa, the *olfactory mucosa*, contains receptors for the sense of smell (see Fig. 7.37). Although the olfactory mucosa is extensive in lower mammals, in man it is confined to a relatively small area in the roof of the nasal cavities. Note the close proximity of the nasal cavities to the orbital cavities **O** and the anterior cranial fossa **ACF**.

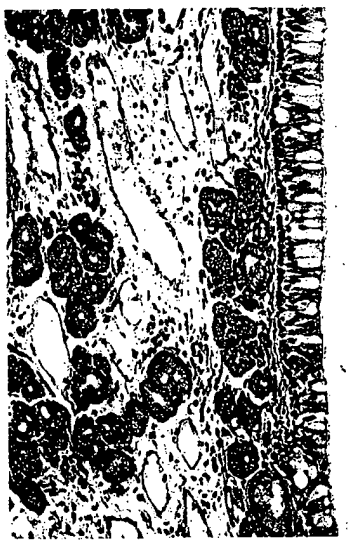

Fig. 11.2 Nasal mucosa

(H & E × 128)

The nasal mucosa consists of a pseudostratified, columnar, ciliated epithelium with numerous goblet cells supported by a richly vascular lamina propria containing serous and mucous glands. These features reflect the protective functions of the nasal mucosa, processes which begin in the nasal cavities and continue throughout the respiratory tract.

Particulate matter in inspired air is trapped in a thin layer of surface mucus secreted by the goblet cells of the surface epithelium and the mucous glands of the lamina propria. Co-ordinated, wavelike beating of cilia propels mucus with trapped particles towards the pharynx where it is swallowed and inactivated in the gastro-intestinal system. The entrance to each nasal cavity, the *nasal vestibule*, is lined by skin which has short, coarse hairs called *vibrissae* which may trap the largest particles before they reach the nasal mucosa.

The temperature of inspired air is adjusted to that of the body by heat exchange between the air and blood flowing in a rich plexus of thin-walled venules in the lamina propria. Inspired air is humidified by the watery secretions of serous glands also located in the lamina propria. A mucosa similar to that of the nasal cavities also lines the nasopharynx, paranasal sinuses and auditory tubes.

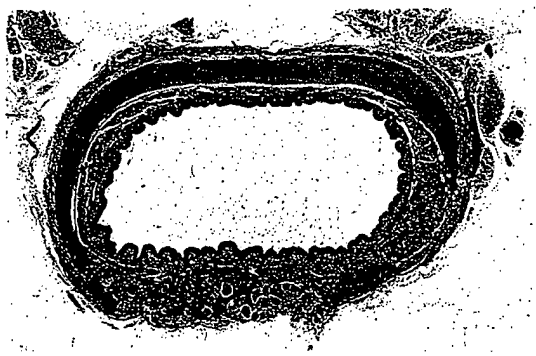

Fig. 11.3 Trachea

(TS: H & E|Alcian blue × 6)

This specimen from a newborn child shows the general structure of the trachea. The trachea is a flexible tube of fibro-elastic connective tissue and cartilage which permits expansion in diameter and extension in length during inspiration, and passive recoil during expiration. A series of C-shaped rings of hyaline cartilage **C**, stained blue in this preparation, support the tracheal mucosa **M** and prevent its collapse during inspiration. Bands of smooth muscle, called the *trachealis muscle* **T**, join the free ends of the rings posteriorly; contraction of the trachealis reduces tracheal diameter and thereby assists in raising intrathoracic pressure during coughing. A few strands of longitudinal muscle are disposed behind the trachealis muscle.